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(54) Title: DRUG DISCOVERY USING GENE EXPRESSION PROFILING

(57) Abstract: The invention relates to a method of determining a candidate condition that is treatable with a chemical composition by (1) providing a relational database comprising phenotype data values and gene expression profile data values, wherein each of the gene expression profile data values is related to at least one phenotype data value, and each of the phenotype data values is associated with a condition in an individual; (2) contacting a cell with the chemical composition; (3) obtaining a test gene expression profile of the cell after the contacting; (4) querying the relational database with the test gene expression profile to obtain a test phenotype; and (5) determining the candidate condition associated with the test phenotype.

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DRUG DISCOVERY USING GENE EXPRESSION PROFILING

5

Background of the Invention

DNA chips allow relatively quick and easy generation of a gene expression profile in a particular cell or tissue. See, e.g., Southern et al., Trends Genet. 12:110-115, 1996; and Ginot, Human Mutation 10:1-10, 1997. Additional aspects of DNA microarrays and drug development are discussed in De SaiZieu et al., Nature Biotechnology 16:45-48, 1998; Heller et al., Proc. Natl. Acad. Sci. USA 94:2150-2155, 1997; Lockhart, Nature Biotechnology 14:1675-1680, 1996; Fields et al., Pro. Natl. Acad. Sci. USA 96:8825-8826, 1999; Lander, Nature Genetics S21:3-4, 1999; and Bowtell, Nature Genetics S21:25-32, 1999.

Summary of the Invention

The invention relates to new methods for finding compositions or compounds that can be used to treat a condition or disease in an individual. These new methods do not require expensive or time-consuming biological assays but instead rely on the generation and analysis of gene expression profiles of different cells under various conditions. DNA chips help facilitate these methods.

Accordingly, the invention features a method of determining a candidate condition (including a disease such as cancer, asthma, osteoporosis, Alzheimer's Disease, and diabetes) that is treatable with a chemical composition by (1) providing a relational database having phenotype data values and gene expression profile data values, each of the gene expression profile data values relating to at least one phenotype data value, and each of the phenotype data values associated with a condition in an individual; (2) contacting a cell with the chemical composition; (3) obtaining a test gene expression profile of the cell after the contacting; (3) querying the relational database with (or using) the test gene expression profile to obtain a test phenotype; and (4) determining the

candidate condition associated with the test phenotype.

In general gene expression profiles of a particular cellular or cell-derived sample can be generated using a combination of standard techniques, such as sequential or
5 parallel Northern blotting, differential display technologies, or nucleic acid microarray technology, which will be discussed below. A phenotype can include any cellular characteristic such as metastatic, apoptotic, necrotic, or normal.

The database can further include cell identity data
10 values, each of the cell identity data values being related to at least one of the phenotype data values and to at least one of the gene expression profile data values.

The test gene expression profile can be obtained by a process including (1) isolating mRNA from the cell; (2) producing
15 cDNA from the mRNA; (3) hybridizing the cDNA to an array of nucleic acid elements (e.g., on a glass or silicon-based support), each element corresponding to a gene; and (4) measuring the extent to which cDNA has bound to each element, thereby determining the test gene expression profile. To facilitate the
20 measuring step, the cDNA can be labeled during or after cDNA synthesis with a label such as a radionuclide, fluorescent molecule, luminescent molecule, or a chromogenic molecule such as an enzyme. The composition in the above method can contain a
25 pure bioactive compound or mixture of a number of pure compounds, or a crude extract including a plant extract or an extract of an animal tissue.

The invention also includes a computer system having (1) a memory storing a database including phenotype data values and gene expression profile data values, each of the gene expression
30 profile data values relating to at least one phenotype data value, and each of the phenotype data values associated with a condition in an individual; (2) an input device configured to provide a test gene expression profile obtained from a cell after contacting the cell with a composition; and (3) a processor
35 configured by a program to query the database using the test gene expression profile to obtain a test phenotype. The computer

system can further include an output device for conveying the test phenotype or the condition associated with the test phenotype. In addition, the database can further include cell identity data values, each of the cell identity data values being
5 related to at least one of the phenotype data values and to at least one of the gene expression profile data values.

The invention also features a computer-readable medium having a program adapted to configure a machine to query a database with (using) a test gene expression profile to obtain a
10 test phenotype, the database including phenotype data values and gene expression profile data values, each of the gene expression profile data values relating to at least one phenotype data value, and each of the phenotype data values associated with a condition in an individual. The database can further include
15 cell identity data values, each of the cell identity data values being related to at least one of the phenotype data values and to at least one of the gene expression profile data values.

Databases useful in the invention can further include condition data values associated with the condition in the
20 individual.

Also featured in the invention is a method of identifying a candidate mixture (e.g., a plant, fungal, bacterial, or animal tissue extract) for treating a condition in an individual, by (1) providing a first gene expression profile of a conditioned cell,
25 the conditioned cell exhibiting a phenotype that can be correlated with the condition in the individual; (2) contacting the conditioned cell with the mixture having a plurality of (e.g., at least 10 or 100) different types of bioactive molecules; (3) determining a second gene expression profile of
30 the conditioned cell after the contacting; and (4) comparing (e.g., by using a computer) the second gene expression profile with the first gene expression profile. A change in the first gene expression profile relative to the second gene expression profile indicates that the mixture is a candidate composition for
35 treating the condition in the individual. A bioactive molecule is a molecule that elicits a biochemical or cellular response in

a cell. Bioactive molecules of the same type have the same molecular structure.

The second gene expression profile can be determined by a process including (1) isolating mRNA from the conditioned cell; (2) producing cDNA (e.g., a labeled cDNA) from the mRNA; (3) hybridizing the cDNA to an array of nucleic acid elements, each element corresponding to a gene; and (4) measuring the extent to which cDNA has bound to each element, thereby determining the second gene expression profile. The array of nucleic acids can be bound to a glass or silicon-based support.

The method of identifying a mixture can further include (1) providing a third gene expression profile of a cell, the cell being conditioned to produce the conditioned cell; and (2) comparing the second gene expression profile with the third gene expression profile. The greater the similarity between the second gene expression profile and the third gene expression profile, the greater the likelihood that the candidate mixture can treat the condition in the individual. By "conditioned" is meant any process which biologically, physiologically, genetically, or biochemically alters at least one characteristic of a cell. For example, exposing a normal cell to ionizing radiation to induce immortal growth is a type of conditioning. In this example, the alteration is likely to be genetic, as well as physiological. Alternatively, the conditioned cell can exhibit a normal phenotype, while the cell that is condition is cancerous. This can be achieved by introduction of a vector expressing an anti-oncogene (e.g., p53) into a cell to condition it.

In addition, the invention includes a method of identifying a candidate compound for treating a condition in an individual by (1) providing a first gene expression profile of a conditioned cell, the conditioned cell exhibiting a phenotype that can be correlated with the condition in the individual; (2) contacting the conditioned cell with a mixture; (3) determining a second gene expression profile of the conditioned cell after the contacting; and (4) comparing the second gene expression profile

with the first gene expression profile. A change in the first gene expression profile relative to the second gene expression profile indicates that the mixture contains the candidate compound for treating the condition in the individual.

5 This method can further (1) providing a third gene expression profile of a cell, the cell being conditioned to produce the conditioned cell; and (2) comparing the second gene expression profile with the third gene expression profile. The greater the similarity between the second gene expression profile
10 and the third gene expression profile, the greater the likelihood that the candidate compound can treat the condition in the individual. Once these additional steps are performed, the method can include (1) fractionating the mixture to obtain a fraction; (2) contacting the conditioned cell with the fraction;
15 (3) determining a fourth gene expression profile of the conditioned cell after the contacting; and (4) comparing the fourth gene expression profile with the second gene expression profile and the third gene expression profile. A fourth gene expression profile that is more similar to the third gene
20 expression profile than the second gene expression profile indicates that the fraction contains the candidate compound for treating the condition in the individual.

 Alternatively, the method of identifying a candidate compound can further include (1) fractionating the mixture to
25 obtain a fraction; (2) contacting the conditioned cell with the fraction; (3) determining a third gene expression profile of the conditioned cell after the contacting; and (4) comparing the third gene expression profile with the first gene expression profile and the second gene expression profile. A third gene
30 expression profile that is more dissimilar to the first gene expression profile than the second gene expression profile indicates that the fraction contains the candidate compound for treating the condition in the individual.

 In general, each of the gene expression profile data
35 values or gene expression profiles above can include expression levels for at least 10 genes (e.g., at least 100 or 1000 genes).

The upper limit of the number of genes represented in the profile is of course dependent on the estimated total number of genes in the genome of the organism studied. The human genome has been estimated to contain about 100,000 genes.

5 In one aspect, the methods of the invention can be used to test a preexisting purified compound, mixture of compounds, or extracts, each currently without any pharmaceutical use, for its ability to alter gene expression in a cell in a manner consistent with a condition or disease. No biological assays are required
10 for this analysis, though they can be included as confirmatory assays. This process, because it begins with a purified compound and looks for a disease to treat, is hereby termed "reverse drug discovery."

In another aspect, the methods of the invention also
15 allow testing of complex mixtures, such as extracts, containing numerous molecules for efficacy against a condition or disease in a patient, again without any biological assays required. These methods can be used as a first screen of complex mixtures or to validate mixtures believed to have potential pharmaceutical
20 utility. Subsequently, the mixtures can be fractionated using standard techniques such as chromatography, and each of the fractions tested in the methods of the invention.

Other features or advantages of the present invention will be apparent from the following drawings and detailed
25 description, and also from the claims.

Detailed Description of the Invention

The methods and various computer-related aspects of the invention rely on the use of gene expression profiles or a
30 database thereof in finding a condition or disease that is treatable in an individual. In other words, known purified compounds, with or without known pharmaceutical uses, can be screened to determine possible use in treating any condition that can be represented in a gene expression profile.

35 The advent of DNA chips and other arrays has greatly accelerated and simplified the acquisition of gene expression

profiles from biological samples. Therefore, the use of DNA arrays and associated tools can be used with the methods of the invention. A relatively comprehensive discussion of arrays can be found in Bowtell et al., supra, which is summarized below.

- 5 Bowtell et al. and references cited therein are hereby incorporated into this document.

I. Generation of RNA from Biological Samples to Be Profiled

- 10 RNA can be isolated from almost any abundant biological sample using standard protocols or commercially available kits. However, care must be taken in scrupulously identifying and harvesting the cells from which RNA is to be isolated. Mistaken identification can result in corrupting both the specific expression profile for that cell type and the gene expression
15 profile database to which the specific profile belongs. One means of ensuring purity of the cell sample is to purchase or order a tissue or cell sample from a depository, such as the American Type Culture Collection.

- Although large numbers of archival samples are
20 available in many clinical departments, often the samples are sub-optimal with respect to RNA integrity, fixation, or critical patient information. The establishment of suitable tissue banks is a logical adjunct to any in-depth RNA analysis of human tissue; repositories must address issues of appropriate
25 collection and storage and also ensure that the samples are accompanied by appropriate patient information, including treatment, outcome, epidemiological and family history data. The National Cancer Institute (NCI) coordinates a centralized tumor bank for North American researchers ([http://www-](http://www-chtn.ims.nci.nih.gov/)
30 [chn.ims.nci.nih.gov/](http://www-chtn.ims.nci.nih.gov/)). Commercial tissue banks, such as LifeSpan BioSciences, also provide access to a wide variety of human disease tissues (<http://www.lsbio.com/>).

- Diseased tissue generally contains a mixture of normal tissue, inflammatory cells, necrotic tissue and, in cancer
35 samples, areas of different grade. Similarly, healthy tissue also includes a range of cell types. All of these elements can

combine to produce a complex RNA expression profile.

Microdissection capability is thus critical for microarray studies involving tissues and is also useful for associated technologies such as comparative genomic hybridization. Current

5 protocols for fluorescent labelling of RNA demand large quantities of RNA, which impedes the use of microdissected RNA on GeneChip⁷ and glass slide arrays. Laser-based microdissection offers a means of more rapidly obtaining pure material than conventional techniques. The commercially available laser
10 capture microdissection microscope (<http://www.arctur.com>) is thus a valuable adjunct to microarray studies. Strategies for using limited material include PCR-amplification of total cDNA before labelling, or the generation of ³³P-labelled nucleic acids (or targets) for filters and glass slides, as these require
15 relatively small amounts of total RNA. Xenografts provide an in vivo means of amplifying limited amounts of tumor cell material and may reduce levels of contaminating non-neoplastic host tissue, although they may fail to recapitulate the expression pattern of the primary tumor.

20 The process of obtaining large amounts of RNA from a homogeneous cell population is greatly simplified when using continuous cell lines. It is important to remember that most microarray analyses do not measure absolute levels of RNA but rather compare RNA levels between two samples. Attention to
25 technical details such as density, pH and possible effects of inducers used in conditional systems is critical.

II. Making and Using Microarrays

Once a suitable RNA preparation is obtained,
30 interrogation of that RNA to produce a gene expression profile can be performed using microarrays.

The complexity of the available arrays remains a major issue and relates to the current state of identification of all the genes in a given organism and the clone sets that house these
35 genes. The complete sequence of the human genome and that of many model organisms are or will be available. Until that time,

however, the sequence of most genomes except those of *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and some unicellular microbes are incomplete.

Traditional approaches to gene discovery such as the
5 cloning of recessive or dominant mutations or the genes encoding
specific proteins have identified approximately 7,000 human
genes. In contrast, large-scale expressed sequence tag (EST)
sequencing has greatly accelerated the rate of gene discovery.
Initially promoted by Craig Venter and associates, EST projects
10 have spread into both the commercial and academic arenas. In
1991, Merck and Washington University established a collaboration
that ultimately fostered the deposition of 480,000 human EST
sequences into GenBank. That number has now grown to over one
million human ESTs, particularly through the efforts of
15 Washington University and members of the IMAGE consortium
(Integrated Analysis of Genomes and their Expression; <http://www-bio.llnl.gov/bbrp/image/image.html>), and more recently, through
those of the Cancer Genome Anatomy Project (CGAP;
http://www.ncbi.nlm.nih.gov/UniGene/gene_discovery.html). EST
20 sequences are deposited in dbEST
(<http://www.ncbi.nlm.nih.gov/dbEST/index.html>), a division of
GenBank, in which an automated process called UniGene compares
ESTs and assembles overlapping sequences into clusters in a
similar manner to shotgun sequencing projects
25 (<http://www.ncbi.nlm.nih.gov/UniGene/index.html>). Some ESTs
correspond with known genes, but the majority represent partially
sequenced novel genes. Ideally each cluster would correspond
with one gene, but as several non-overlapping clusters may exist
for large or low abundance genes, the number of clusters is
30 likely to exceed the number of separate genes from whose sequence
they are derived. Additionally, errors in alignment programs can
produce false clusters (over-clustering). Clone sets, comprising
a single representative of each cluster (usually the most 5'
clone), are sold by licensed vendors ([http://www-bio.llnl.gov/bbrp/](http://www-bio.llnl.gov/bbrp/image/idistributors.html)
35 [image/idistributors.html](http://www-bio.llnl.gov/bbrp/image/idistributors.html)).

The large number of ESTs identified by the Institute for

Genomic Research (TIGR, <http://www.tigr.org/>) are now publically available. TIGR mouse, human, zebrafish, rat and plant clones can be viewed in their respective Gene Indices databases, where they have been assembled into tentative consensus sequences (and

5 can be considered the equivalent of UniGene clusters) by comparison with TIGR and GenBank databases. The American Type Culture Collection provides single human and mouse TIGR clones, including a limited number of clones with the complete open reading frame of known genes

10 (<http://www.atcc.org/hilights/tasc2.html>).

Genome Systems (GS; <http://www.genomesystems.com/>) and Research Genetics (RG; <http://www.resgen.com/>) are the two IMAGE clone vendors with the most developed clone sets. Both GS and RG have undertaken a process of clone validation through restreaking

15 (to isolate single cells) and resequencing, as the original UniGene sets had a significant discrepancy between actual and designated clone sequence and many IMAGE clones were mixed.

In addition to providing individual clones and clone sets, both companies sell filters on which clones or

20 purified DNAs have been arrayed at high density to provide targets for reverse-transcribed probes and supply some clone sets both as bacterial colonies in microtitre plates and as PCR products. The latter have the advantages of avoiding both the risk of T1 phage contamination and the need to isolate plasmids

25 for PCR, a step some labs feel is essential to obtain clean DNA for arraying purposes.

GS has been able to add to the human IMAGE clones via its access to additional human cDNA from Incyte

(<http://www.incyte.com>), an organization that has perhaps

30 sequenced more human cDNA (3 million) than any other. Access to the Incyte LifeSeq database is currently limited to approximately 25 pharmaceutical partners (no academic institutions have subscribed). Of the human clones in LifeSeq, 2.3 million are Incyte-proprietary. By using the Incyte clone set, GS has

35 recently produced a sequence-verified set of 9,844 human clones that includes many known genes present in the UniGene set (about

5,000 of about 7,400). The GS resequenced human clones are also free from low-level T1 contamination present in IMAGE clones, which can be a serious problem (<http://www-bio.llnl.gov/bbrp/image/phage.html>).

5 Both RG and GS clone sets also contain a large number of ESTs. Whether those ESTs present are likely to be expressed in your favorite tissue or cell line is hard to predict. Little or no information is provided concerning the basis for selection of ESTs; they appear to represent clones from a range of libraries
10 with no preselection on the basis of biological interest. That situation is changing as the focus of the Washington University human EST project shifts to CGAP clones and companies provide clones that have interesting expression patterns.

EST sequences of other organisms, such as mouse, rat,

15 *Drosophila melanogaster*, and *Arabidopsis Thaliana*, have accumulated at different rates. A limitation of the mouse EST project (http://genome.wustl.edu/est/mouse_esthmpg.html) is that sequencing has been carried out from the 5' end of cDNA. As the length of the 5' ends of cDNA is variable, the number of clusters
20 obtained is greater than if oligo(dT)-primed cDNA had been sequenced from the 3' end. As a result of this, and because fewer mouse cDNAs have been sequenced and clusters are smaller, the proportion of novel genes in the current mouse clone sets is substantially fewer than in the human sets. Both RG and GS are
25 performing 3' resequencing of a collection of mouse clones. These clones have been selected because they either correspond to known mouse genes or because they appear to be related to other genes of interest, thus effectively collapsing some of the earlier clustering. Celera (<http://www.celera.com/>), a
30 commercial offshoot of TIGR, is sequencing the *Drosophila* genome as a prelude to their human genome sequencing project; the *Drosophila* sequence also should be ready soon.

Obtaining the entire genomic sequence of *S. cerevisiae* allowed a near-complete set of genes to be generated by PCR,
35 which have been arrayed and analyzed. Although it will be more difficult to identify coding sequences in more complex organisms,

the convergence of genome and EST sequencing projects in the near future will ensure the identification of non-redundant clone sets that encompass all genes for a variety of other species.

Filter arrays have the advantage of being relatively
5 affordable and needing no special equipment to use, although potential users should be aware that large format phosphorimager screens may be required with larger filters. Filters are also useful for scarce RNA (for example from microdissected tissue), as only approximately 50 ng of total RNA is required for a single
10 experiment (100 μ g of RNA is typically required for a fluorescent probe). The major disadvantage of filters is that comparison of expression between two samples requires hybridization of each sample to separate duplicate filters, or to a single filter that must be stripped and hybridized sequentially. The sensitivity of
15 lysed colony filter arrays is reported to be limited to high- and medium-abundance genes. In contrast, hybridization of fluorescently labelled nucleic acids to slide arrays or gene chips can detect low abundance genes, an important point as most genes fall within this category. Direct comparison of
20 GeneChip7, slide, and filter arrays is required, however, to settle the considerable debate concerning the relative sensitivity of filters hybridized with 32 P-labelled targets versus GeneChip7 or slides hybridized with fluorescent targets. Commercial filter arrays of clone sets are available from
25 Clontech, GS, and RG.

Important considerations in the choice of array include whether the clones used to produce the arrays are restreaked and sequence-verified, whether DNA or lysed colonies are arrayed, and the number of known genes and ESTs. Clontech filters only
30 include known genes, preselected and grouped for their involvement in specific processes such as apoptosis. Current GS filters use lysed bacterial colonies, whereas purified DNA is arrayed on both Clontech and RG filters. The lower complexity and higher purity of arrayed DNA is thought to increase the
35 sensitivity of these filters. GS is expected to release sequence-verified human arrays that include a large proportion of

known genes present in the UniGene set (approximately 5,000).

GeneChip⁷ arrays and commercially available glass slides are at the more sophisticated end of microarray analysis and are extremely suitable for use in the methods of the invention. At present, options include Affymetrix GeneChip⁷ arrays (<http://www.affymetrix.com>) and slide arrays from Incyte (which has recently acquired Synteni (<http://www.synteni.com/>)).

Incyte does not sell slide arrays as such but provides a service whereby samples applied to slides and the data returned.

Molecular Dynamics and Clontech have recently announced that they will also provide slide arrays (<http://www.mdyn.com/>). Genometrix (www.genometrix.com) provides custom synthesis of large numbers of low complexity arrays (up to several hundred probes). Using a proprietary method for arraying oligonucleotides, they mass-produce slides at low cost (approximately \$10/array for orders of 1,000-10,000 individual arrays) and are developing devices for high-throughput analysis of these arrays.

Hyseq (<http://www.hyseq.com/>) have developed a novel method where hybridization of DNA targets with all possible pentamer or heptamer oligonucleotides allows inference of sequence from the pattern of oligonucleotide hybridization. This strategy has been applied to measuring the abundance of individual cDNA in libraries from tissues of interest, thereby providing an estimate of individual gene expression. Hyseq offers this type of analysis in house.

The first glass slide arrays were produced in Dr. Pat Brown's laboratory at Stanford University (<http://cmgm.stanford.edu/pbrown/index.html>) and from there the technology spread. Brown's web site also contains detailed specifications for building an arrayer and associated software. Additional protocols and some hardware details are available at http://chroma.mbt.washington.edu/mod_www/.

Several companies have produced arrayers for sale. Each is a relatively simple XYZ axis robot that can position the print head with a similar degree of precision. Critical determinants

when choosing among them are whether the machine has a proven track record in the field, the technical support network available, cost, ease of use, capacity, features such as bar code reader, temperature control, plate stacker, microtitre plate lid remover, and pen design.

Beecher Instruments was started by one of the engineers who developed the robot used by the NHGRI; it now sells an equivalent arrayer and reader, which has the advantage of having been successfully field tested for more than two years. The BioRobotics microGrid (<http://www.biorobotics.co.uk/>) combines many features into a compact machine. Initially designed for robotic gridding of clones from 96- or 384-well microtitre plates onto filters, it can also replica-plate libraries and be upgraded to print glass slides and filters and re-array bacterial libraries (also known as cherry picking). Genomic Solutions (www.genomicsolutions.com) produces a complete system of arrayer, hybridization station, reader, and analysis software. Genetic Microsystems (<http://www.geneticmicro.com/>) also produce a relatively affordable machine. Molecular Dynamics conducts a Microarray Technology Access Program (MTAP), where participants gain early access to microarray technology developed by Molecular Dynamics and Amersham. Molecular Dynamics produces arrayers for MTAP participants.

One of the most important factors affecting the performance of the arrayer are the shape, reproducibility, and durability of the pens (also referred to as tips, pins, and quills). Uneven pens deliver unequally during a print run and tax the abilities of image analysis programs. Precision tips are available from several suppliers, including Beecher Instruments, Majer Precision Engineering (<http://www.majerprecision.com>), who custom-machine high-precision pens from a range of materials, and Telechem International (<http://www.wenet.net/~telechem/>), who also offer related microarray equipment and consumables.

Filters are hybridized with ³³P-labelled probes and signal is detected using phosphorimager screens. Phosphorimager systems

are produced by Molecular Dynamics, Packard Instrument, and Fuji.

The Packard Cyclone instrument is relatively low in cost but offers a high degree of resolution for array work. Analyses can be carried out by eye, by sending a GIFF data file via the Web to a company to be read, or using commercial or public domain software (see below).

The Affymetrix fluorescence reader, produced by Hewlett Packard, is currently customized for GeneChip7 arrays. Hewlett Packard plans to build readers capable of reading both GeneChip7 and glass slides. General Scanning (<http://www.genscan.com/>) released the ScanArray 3000, a compact scanning confocal laser, and Beecher Instruments sells a reader based on the machines used at NHGRI and the National Cancer Institute (NCI). Like the arrayer, the Beecher reader is not supported by a service network but its high degree of sensitivity has provided a benchmark for other commercial readers. Molecular Dynamics have recently released the Avalanche reader, which is based on one developed during the MTAP program.

The above readers are laser confocal scanning devices, except for the Genomic Solutions reader, which uses a CCD camera and filter blocks, facilitating upgrades to reading different fluorophors. Direct comparison would be very useful and can be carried out in the context of the methods of the invention.

III. Data Analysis

A typical array experiment generates thousands of data points. Informatics can be categorized as either >tools= or >analyzers=. Tools include software that operate arraying devices and perform image analysis of data from readers, databases to hold and link information, and software that link data from individual clones to Web databases. Some involve fairly straightforward software but are nevertheless quite extensive. The Brown laboratory has made available software for operating custom built arrayers (<http://cmgm.stanford.edu/pbrown/mguide/software.html>).

The quality of image analysis programs is crucial for accurate interpretation of signals for slide and filters. Dr. Yidong Chen (NHGRI) has developed a sophisticated image analysis program for slides and filters, deArray, that is available but not supported (www.nhgri.nih.gov/DIR/LCG/15K/HTML/). Mark Boguski and colleagues have developed software that is capable of both analyzing microarray data and linking to databases such as Entrez and UniGene, and this can be downloaded from the web (www.nhgri.nih.gov/DIR/LCG/15K/HTML/).

Commercial readers and arrayers provide software for data analysis: Synteni have developed a sophisticated program for analyzing microarray data (GemTools); RG sells the Pathways package to analyze their filters; and the Visage suite can be purchased from Genomic Solutions, separate from their hardware. Silicon Genetics (<http://www.sigenetics.com>) provides the GeneSpring package for analyzing data from Affymetrix GeneChip7 and other microarray experiments.

RNA expression analysis represents only one parameter by which cells or tissues may be characterized. Depending on the experiment, epidemiological or molecular pathological data, genomic changes (gains or losses) or sensitivity to drugs may be additional parameters that will influence the interpretation of microarray data. The ability to combine RNA and protein expression data to comprehensively profile both transcriptional and post-transcriptional changes in cells and tissues is particularly appealing, although the number of proteins that can be profiled at this stage is substantially less than the number of genes. Although it is more difficult to identify proteins that are differentially expressed, techniques for rapid and reproducible two-dimensional gel protein separation and mass spectrometry-based protein identification make high-throughput proteomics a highly desirable adjunct to microarray RNA expression analysis. Thus, the methods of the present invention can include proteomic assays.

Without further elaboration, it is believed that one skilled in the art can, based on the above disclosure and the

description below, utilize the present invention to its fullest extent. The following detailed description is to be construed as merely illustrative of how one skilled in the art can practice the invention and is not limitative of the remainder of the disclosure in any way. Any publications cited in this disclosure are hereby incorporated by reference.

A pattern of gene expression is obtained from normal cells, diseased cells, and compound-treated cells using DNA chips. The gene expression pattern is a representation of the state of the cell in response to the disease or to treatment. Comparison of gene expression by healthy, diseased, and treated cells will in principle reveal patterns of gene expression that are diagnostic for therapeutic as opposed to pathological effects. Once validated, such patterns can be used to screen complex compound mixtures for molecules with desirable properties. This approach does not depend on knowing the precise molecular mechanism of a disease; rather, it identifies sets of genes as diagnostic for a disease state, without requiring a specific knowledge of the contribution of the genes to disease.

Since the compounds identified from screening the complex mixtures are totally based on the change of expression pattern, the pattern recognition can be accomplished via bioinformatic analysis. Results from this analysis can then indicate the type of disease to be treated. Therefore, this invention will be most useful in discovering drug leads for treating various human pathologies; from cardiovascular to autoimmune disorder; from infectious disease to cancer.

After the completion of human genome sequencing, the invention will further allow us to identify groups of genes that may share common regulatory elements from a single given DNA chip experiment. This invention can thus provide a revolutionary approach towards novel lead drug discovery against so far unknown groups of genes that may share a genuine disease-relevant common elements.

As described within this document, the mixture or composition can include a plant extract, microbial broth, or

chemical library. Fractionation is defined in two ways. If a mixture is from natural extract the fractions can be obtained from column chromatography. If a mixture is a synthetic chemical library from combinatorial synthesis, the sub-libraries (also
5 named fractions for simplicity) will then be prepared using the appropriate combinatorial synthesis. Diseased cells are defined as a biopsy or a cell line, either taken from diseased tissue or treated with a defined stimulus.

The gene expression fingerprints of some selected drugs
10 on appropriate disease tissue or microorganisms with the DNA chips are compiled in a relational database to provide basis for pattern recognition of a given drug. A common characteristic pattern of structurally similar drugs usually indicates that these drugs may work through a similar mechanism to exert their
15 drug effect. This common pattern can be used to identify new drug leads using the pattern recognition analysis. This will allow discovery of new drug leads with new chemical structures. This new lead may serve as a basis for further structural modification for improved pharmacokinetics, including decreased
20 side-effects.

For primary screening on DNA chips, a sample of the disease cells is treated with mixtures for a specified time period. After the treatment the mixture is washed away and the cells are lysed. Fluorescently-tagged cDNA is prepared by
25 reverse transcription of mRNA from both experimental samples. After hybridization with the DNA chips and appropriate washing steps, images are analyzed via laser scanning. A controlled sample without treatment is also conducted with the same procedure. The housekeeping genes are included in the expression
30 system for the purpose of a quantitative measurement. The fluorescence intensity ratios for the control vs. the test samples are determined, and the changes in gene expression are compared. Mixtures that produce a change of gene expression profiles are selected for secondary screening.

35 For secondary screening, fractionation is performed on the selected screening mixtures obtained from the primary

screening. Aliquots of the disease cells are treated separately with each fraction. Gene expression profiles of the fraction-treated cells are compared with those tabulated in the primary screen. Comparison of the expression data obtained from the primary and the secondary screens may require further screens with selected sub-fractions until active components are identified. Alternatively, gene expression-altering activity may rely on a complex fraction, in which case no further fractionation is possible without decreasing or eliminating the mixture's bioactivity.

At some points in the procedure (e.g., during the sub-fraction screening), the gene expression pattern may indicate an additive effect between different fractions. Further analysis using combination screens of fractions could then be employed to identify multiple agents that exhibit the combined additive effect.

The genomes of several microorganisms have been completely sequenced, allowing for comprehensive analysis of gene expression under different conditions. DNA chips representing the genome of a given microbe (e.g., a bacterium, fungus, or yeast) are used to probe the gene expression profile in different growth conditions: free-living, host-associated, or with or without drug treatment. Since many genes required for host infection are expressed preferentially by microbes in the process of infection itself, DNA chips allows rapid identification of potential drug targets. Lead compounds can be evaluated simultaneously for impact on gene expression by both the infecting microbe and the host, thereby allowing the identification of potential side effects in parallel with an assessment of therapeutic efficacy.

Procedures similar to the primary and secondary screens as described above can be applied to discover drug leads for treating microorganisms.

A reference set of gene expression patterns are assembled for a given microorganism in the free-living state, in association with the infecting host, and if available, in

infected hosts treated with known drugs. Comparison of these patterns will allow the identification of potential targets and assessment of mixture effects on these targets.

Labeled cDNA probes are prepared from microbes cultured in vitro or isolated from an infected host, in the presence or absence of the compound mixtures. Changes in gene expression profiles in response to a given mixture are recorded and the mixture itself is selected for further fractionation. Fractions are evaluated for their ability to generate changes in gene expression. Fractions that fail to modify the pathogenic expression profile are discontinued, whereas fractions that reproduce changes in the profile are sub-fractionated. This process is repeated until candidate structures that are responsible for the change of the expression pattern can be identified. In some cases, of course, the activity will be dependent on a mixture of compounds.

Bioinformatic analysis is often used to decipher the compiled expression data with regard to the gene expression fingerprint library established by the above procedures. Two scenarios are expected from the analysis.

Scenario 1: Fractions or compounds that exhibit similar expression pattern to that of the known drug-treated cell may indicate that these fractions or compounds intervene the cellular process through a similar biological mechanism. However, the discovered drug lead with new chemical structure may serve as a basis for further structural modification for improved pharmacokinetics, thereby decreasing the potential side-effects of the drug.

Scenario 2: If a fraction or compound shows a completely different expression pattern than that from the known drug treated tissue sample, the fraction or compound may serve as a new drug lead for a novel drug target with a new mode of action in intervening the disease process. This scenario will provide novel strategies in discovering drug leads for so

far unknown drug targets with a completely unknown drug action.

The structures of the identified compounds are characterized using conventional instrumentation analysis.

5 Structures that are amenable to organic synthesis will be prepared and scaled up in the laboratory for both in vitro and in vivo testing in an established disease tissue, disease animal model, intact microorganism, or infected tissue. Once the lead drug is validated, medicinal or combinatorial chemistry can be
10 applied to conduct optimization of the drug lead into a clinically useful drug candidate.

As opposed to conventional drug development, the natural extract or combinatorial libraries used as described in this invention for drug lead discovery can provide another opportunity
15 to discover a desirable disease treatment method using a selected combination therapy. This can be discovered through the observation of the combined effect of different fractions on gene expression patterns which are considered most desired in the disease treatment.

20 There are two criteria that must be met by cell lines to be useful in a microarray-based screening assay. First, the cell lines should individually or as a set be able to model physiologically normal and disease states. Secondly, it should be possible to isolate sufficient mRNA to generate fluorescently-
25 labeled cDNA (approximately 10 μ g, which is typically isolated from 5×10^6 cells).

An example of such an analysis for rheumatoid arthritis (RA) was recently reported (Heller et al., Proc. Natl. Acad. Sci. USA 94:2150, 1996). The human chondrosarcoma cell SW1353
30 releases matrix-degrading metalloproteinases (MMPs) when treated with TNF α or IL-1. Since TNF α production contributes to joint destruction in RA, the gene expression profile of TNF α -induced SW1353 cells should be reflective of the disease state. Comparison of the profiles from uninduced and TNF α -induced SW1353
35 cells could therefore generate a diagnostic gene expression profile. TNF α -induced SW1353 cells would then be treated with

compound mixtures for screening purposes.

Adherent cells are grown in flasks to generate a least 5 x 10⁶ cells per flask. For each preparation of mRNA from TNF α -induced cells treated with a mixture, there would need to be an equivalent amount of mRNA from untreated TNF α -induced cells to serve as a reference. The two sets of mRNA are used to generate cDNA by reverse transcription. The reverse transcription reactions contain dCTP labeled with either of fluorescent dyes Cy3 or Cy5 (Amersham Pharmacia), which results in the generation of fluorescently-labeled cDNA probes. Cy3 and Cy5 have similar excitation spectra but distinct emission spectra. The two sets of cDNAs are combined and hybridized to one or more DNA chips. After washing to remove unbound probe, the chip is analyzed by laser scanning. Since Cy3 and Cy5 have different emission spectra, the amount of each probe hybridized to a given nucleic acid (corresponding to a known gene) on the chip can be quantitated separately, and the ratio of the two signals will indicate whether expression of the gene has changed as a result of exposure to the mixture. Several companies provide software packages that allow for the compilation of gene expression profiles from microarray data (e.g. GeneSight, from BioDiscovery, Inc., Los Angeles, CA). For purposes of screening, any change in mixture-treated cells compared to untreated cells is indicative that the mixture can be used to treat a condition, but particular emphasis will be given to mixtures that reverse the effect of TNF α induction.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of this invention.

For example, instead of determining a gene expression profile associated with a condition or disease de novo, known

gene expression profiles can be used. For example, profiles for multiple sclerosis lesions and corresponding normal tissue is known (Whitney et al., Ann. Neurol. 46:425-428, 1999). In addition, profiles for young and aged skeletal muscle are also
5 available (Lee et al., Science 285:1390-1393, 1999).

What is claimed is:

1. A method of determining a candidate condition that is treatable with a chemical composition, the method comprising providing a relational database comprising phenotype data values and gene expression profile data values, wherein each of the gene expression profile data values is related to at least one phenotype data value, and each of the phenotype data values is associated with a condition in an individual;

contacting a cell with the chemical composition;

obtaining a test gene expression profile of the cell

after the contacting;

querying the relational database with the test gene expression profile to obtain a test phenotype; and

determining the candidate condition associated with the test phenotype.

2. The method of claim 1, wherein the database further comprises cell identity data values, each of the cell identity data values being related to at least one of the phenotype data values and to at least one of the gene expression profile data values.

3. The method of claim 1, wherein the test gene expression profile is obtained by a process comprising

isolating mRNA from the cell;

producing cDNA from the mRNA;

hybridizing the cDNA to an array of nucleic acid elements, each element corresponding to a gene; and

measuring the extent to which cDNA has bound to each element, thereby determining the test gene expression profile.

4. The method of claim 3, wherein the cDNA is labeled.

5. The method of claim 3, wherein the array is bound to a glass support.

6. The method of claim 3, wherein the array is bound to a surface of a silicon-based material.

7. The method of claim 1, wherein the chemical composition contains a pure compound or a mixture of a number of pure compounds.

8. The method of claim 1, wherein the chemical composition contains a plant extract.

9. The method of claim 1, wherein the chemical composition contains an extract of an animal tissue.

10. The method of claim 1, wherein each of the gene expression profile data values includes expression levels for at least 10 genes.

11. The method of claim 10, wherein each of the gene expression profile data values includes expression levels for at least 100 genes.

12. The method of claim 11, wherein each of the gene expression profile data values includes expression levels for at least 1000 genes.

13. A method of identifying a candidate mixture for treating a condition in an individual, the method comprising providing a first gene expression profile of a conditioned cell, wherein the conditioned cell exhibits a phenotype that can be correlated with the condition in the individual;

contacting the conditioned cell with a mixture comprising a plurality of different types of bioactive molecules;

determining a second gene expression profile of the conditioned cell after the contacting; and

5 comparing the second gene expression profile with the first gene expression profile,

wherein a change in the first gene expression profile relative to the second gene expression profile indicates that the mixture is a candidate mixture for treating the condition in the
10 individual.

14. The method of claim 13, wherein the mixture is a plant extract.

15 15. The method of claim 13, wherein the mixture is an extract from an animal tissue.

16. The method of claim 13, wherein the second gene expression profile is determined by a process comprising
20 isolating mRNA from the conditioned cell;
producing cDNA from the mRNA;
hybridizing the cDNA to an array of nucleic acid elements, each element corresponding to a gene; and
measuring the extent to which cDNA has bound to each
25 element, thereby determining the second gene expression profile.

17. The method of claim 16, wherein the cDNA is labeled.

18. The method of claim 16, wherein the array is bound
30 to a glass support.

19. The method of claim 16, wherein the array is bound to a surface of a silicon-based material.

35 20. The method of claim 13, further comprising

providing a third gene expression profile of a cell,
wherein the cell is conditioned to produce the conditioned cell;
and

5 comparing the second gene expression profile with the
third gene expression profile,

wherein the greater the similarity between the second
gene expression profile and the third gene expression profile,
the greater the likelihood that the candidate mixture can treat
the condition in the individual.

10

21. The method of claim 13, wherein the comparing step
is computer-assisted.

15 22. The method of claim 13, wherein each of the first
and second gene expression profiles includes expression levels
for at least 10 genes.

20 23. The method of claim 22, wherein the first and second
gene expression profile each includes expression levels for at
least 100 genes.

24. The method of claim 23, wherein the first and second
gene expression profile each includes expression levels for at
least 1000 genes.

25

25. The method of claim 13, wherein the plurality is at
least 10 different types of bioactive molecules.

26. A computer system comprising

a memory storing a database comprising phenotype data values and gene expression profile data values, wherein each of the gene expression profile data values is related to at least one phenotype data value, and each of the phenotype data values is associated with a condition in an individual;

an input device configured to provide a test gene expression profile obtained from a cell after contacting the cell with a composition; and

a processor configured by a program to query the database using the test gene expression profile to obtain a test phenotype.

27. The computer system of claim 26, further comprising an output device for conveying the test phenotype or the condition associated with the test phenotype.

28. The computer system of claim 25, wherein the database further comprises cell identity data values, each of the cell identity data values being related to at least one of the phenotype data values and to at least one of the gene expression profile data values.

29. A computer-readable medium having a program adapted to configure a machine to query a database using a test gene expression profile to obtain a test phenotype, wherein the database comprises phenotype data values and gene expression profile data values, each of the gene expression profile data values relating to at least one phenotype data value, and each of the phenotype data values associated with a condition in an individual.

30. The computer-readable medium of claim 29, wherein the database further comprises cell identity data values, each of the cell identity data values being related to at least one of the phenotype data values and to at least one of the gene expression profile data values.

31. The method of claim 1, wherein the relational database further comprises condition data values associated with the condition in the individual.

5

32. The method of claim 2, wherein the relational database further comprises condition data values associated with the condition in the individual.

10

33. The computer system of claim 26, wherein the database further comprises condition data values associated with the condition in the individual.

15

34. The computer system of claim 28, wherein the database further comprises condition data values associated with the condition in the individual.

20

35. The computer-readable medium of claim 29, wherein the database further comprises condition data values associated with the condition in the individual.

25

36. The computer-readable medium of claim 30, wherein the database further comprises condition data values associated with the condition in the individual.

37. A method of identifying a candidate compound for treating a condition in an individual, the method comprising

providing a first gene expression profile of a conditioned cell, wherein the conditioned cell exhibits a phenotype that can be correlated with the condition in the individual;

5 contacting the conditioned cell with a mixture;
 determining a second gene expression profile of the conditioned cell after the contacting; and
 comparing the second gene expression profile with the first gene expression profile,

10 wherein a change in the first gene expression profile relative to the second gene expression profile indicates that the mixture contains the candidate compound for treating the condition in the individual.

15 38. The method of claim 37, further comprising providing a third gene expression profile of a cell, wherein the cell is conditioned to produce the conditioned cell; and

 comparing the second gene expression profile with the
20 third gene expression profile,

 wherein the greater the similarity between the second gene expression profile and the third gene expression profile, the greater the likelihood that the candidate compound can treat the condition in the individual.

25

 39. The method of claim 38, further comprising

fractionating the mixture to obtain a fraction;
contacting the conditioned cell with the fraction;
determining a fourth gene expression profile of the
conditioned cell after the contacting; and

5 comparing the fourth gene expression profile with the
second gene expression profile and the third gene expression
profile,

 wherein a fourth gene expression profile that is more
similar to the third gene expression profile than the second gene
10 expression profile indicates that the fraction contains the
candidate compound for treating the condition in the individual.

40. The method of claim 37, further comprising
fractionating the mixture to obtain a fraction;
15 contacting the conditioned cell with the fraction;
determining a third gene expression profile of the
conditioned cell after the contacting; and
 comparing the third gene expression profile with the
first gene expression profile and the second gene expression
20 profile,

 wherein a third gene expression profile that is more
dissimilar to the first gene expression profile than the second
gene expression profile indicates that the fraction contains the
candidate compound for treating the condition in the individual.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/26050

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A01N 63/00; G01N 33/48 US CL : 424/93.7; 702/19 According to International Patent Classification (IPC) or to both national classification and IPC																										
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/93.7; 702/19 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Extra Sheet.																										
C. DOCUMENTS CONSIDERED TO BE RELEVANT																										
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																								
Y,P	US 6,114,114 A (SEILHAMER et al.) 05 September 2000, see especially cols 7-21 and 77-82.	1-40																								
Y,P	WO 9957130 A1 (GENE LOGIC, INC.) 11 November 1999, see especially pages 1-35 and 49-55.	1-40																								
Y,P	WO 00/29846 A2 (CURAGEN CORPORATION) 25 May 2000, see especially pages 62-83.	1-25																								
Y	WO 99/10536 A1 (YALE UNIVERSITY) 04 March 1999, see especially pages 5-86 and 5-68.	1-25																								
Y	WO 99/01581 A1 (GENZYME CORPORATION) 14 January 1999, see especially pages 4-10 and 23-25.	1-25																								
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																										
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>*T</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>*A</td> <td>document defining the general state of the art which is not considered to be of particular relevance</td> <td></td> </tr> <tr> <td>*B</td> <td>earlier document published on or after the international filing date</td> <td>*X</td> </tr> <tr> <td>*L</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>*O</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td>*Y</td> </tr> <tr> <td>*P</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td></td> <td></td> <td>*A</td> </tr> <tr> <td></td> <td></td> <td>document member of the same patent family</td> </tr> </table>			* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A	document defining the general state of the art which is not considered to be of particular relevance		*B	earlier document published on or after the international filing date	*X	*L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*O	document referring to an oral disclosure, use, exhibition or other means	*Y	*P	document published prior to the international filing date but later than the priority date claimed	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art			*A			document member of the same patent family
* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																								
*A	document defining the general state of the art which is not considered to be of particular relevance																									
*B	earlier document published on or after the international filing date	*X																								
*L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																								
*O	document referring to an oral disclosure, use, exhibition or other means	*Y																								
*P	document published prior to the international filing date but later than the priority date claimed	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																								
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		document member of the same patent family																								
Date of the actual completion of the international search 13 NOVEMBER 2000		Date of mailing of the international search report 03 JAN 2001																								
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer <i>Shubo "Joe" Zhou</i> SHUBO "JOE" ZHOU Telephone No. (703) 308-0196																								

INTERNATIONAL SEARCH REPORT**International application No.**
PCT/US00/26050**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99/10535 A1 (YALE UNIVERSITY) 03 March 1999, see especially pages 4-18 and 62-63.	1-25

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/26050

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/26050

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG, MEDLINE, EAST

search terms: Huang, (gene expression profile), (disease or condition), databases

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-12, and 26-36.

Group II: Claims 13-25, and 37-40.

Groups I and II are two distinct inventions because the invention of Group I is directed to methods and computer system/medium for determining a candidate condition mostly by looking for **similarities** between gene expression profile of cells contacted with certain chemical composition and that of any phenotype associated with certain condition in a relational database whereas invention of Group II is directed to methods of identifying a candidate mixture for treating a condition by looking for **differences** in gene expression profile of cells treated with certain mixture from that of a phenotype/condition in the database. Therefore, the Special Technical Feature is different between the above two Groups.